AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph beginning on page 10, line 10, with the following:

The compositions provided herein can further comprise a therapeutic agent (e.g., a cytokine, an anti-cancer agent, an adjuvant, etc.). In some embodiments the adjuvant is alum, monophosphoryl lipid A, a saponin, an immunostimulatory oligonucleotide, incomplete Freund's adjuvant, complete Freund's adjuvant, ~~montanide~~MONTANIDE, vitamin E, a water-in-oil emulsion[[s]] prepared from a biodegradable oil, Quil A, a MPL and mycobacterial cell wall skeleton combination, ENHANZYN[[^{TM}]], CRL-1005, L-121, alpha-galactosylceramide or a combination thereof.

Please replace the paragraph beginning on page 10, line 27, with the following:

Compositions of PSMA protein multimers including a surfactant are also provided. Such surfactants include ~~Tween~~TWEEN 20, ~~Tween~~TWEEN 80, TRITON~~Triton~~ X-100, dodecylmaltoside, cholic acid, CHAPS and combinations thereof.

Please replace the paragraph beginning on page 10, line 30, with the following:

Also provided are compositions of PSMA protein multimers that comprise a cryoprotectant, an antioxidant, a preservative or a combination thereof. Examples of cryoprotectants include a sugar, a polyol, an amino acid, a polymer, an inorganic salt, an organic salt, trimethylamine N-oxide, sarcosine, betaine, gamma-aminobutyric acid, octapine, alanopine, strombine, dimethylsulfoxide and ethanol. When the cryoprotectant is a sugar the sugar can be sucrose, lactose, glucose, trehalose or maltose. In other embodiments when the cryoprotectant is a polyol the polyol can be inositol, ethylene glycol, glycerol, sorbitol, ~~xylitol~~XYLITOL, mannitol or 2-methyl-2,4-pentane-diol. When the cryoprotectant is an amino acid the amino acid can be Na glutamate, proline, alpha-alanine, beta-alanine, glycine, lysine-HCl or 4-hydroxyproline. When the cryoprotectant is a polymer the polymer can be polyethylene glycol, dextran or polyvinylpyrrolidone. When the cryoprotectant is an inorganic salt the cryoprotectant can be sodium sulfate, ammonium sulfate, potassium phosphate, magnesium sulfate or sodium fluoride. Finally, when the cryoprotectant is an organic salt the organic salt can be sodium

acetate, sodium polyethylene, sodium caprylate, proprionate, lactate or succinate. Examples of antioxidants that are part of these compositions in some embodiments include ascorbic acid, an ascorbic acid derivative, butylated hydroxy anisole, butylated hydroxy toluene, alkylgallate, dithiothreitol (DTT), sodium meta-bisulfite, sodium bisulfite, sodium dithionite, sodium thioglycollic acid, sodium formaldehyde sulfoxylate, tocopherol, a tocopherol derivative, monothioglycerol and sodium sulfite. Ascorbic acid derivatives, in some embodiments, include ascorbylpalmitate, ascorbylstearate, sodium ascorbate and calcium ascorbate, while tocopherol derivatives include d-alpha tocopherol, d-alpha tocopherol acetate, dl-alpha tocopherol acetate, d-alpha tocopherol succinate, beta tocopherol, delta tocopherol, gamma tocopherol and d-alpha tocopherol polyoxyethylene glycol 1000 succinate. Examples of preservatives present in the compositions in some embodiments include benzalkonium chloride, chlorobutanol, parabens, thimerosal, benzyl alcohol and phenol.

Please replace the paragraph beginning on page 27, line 32, with the following:

Fig. 13 depicts the cloning protocol for IgG1 antibody cloning into pcDNA. The first four primers shown are set forth as SEQ ID NOs: 34-37, respectively, and the first two amino acid sequences shown are set forth as SEQ ID NOs: 38 and 39, respectively.

Please replace the paragraph beginning on page 29, line 22, with the following:

FIG. 35 shows the results of the comparison of the fully human anti-PSMA antibodies 4.40.1, 4.49.1, 051 and 006 and the murine anti-PSMA antibody 3.9 performed using ~~Biacore~~BIACORE analysis.

Please replace the paragraph beginning on page 37, line 7, with the following:

In some cases where a high salt concentration is used to promote or preserve PSMA dimerization, the salt concentration can be diluted to within a physiologically acceptable range suitable for parenteral use prior to administration. As an example, the salt concentration can be diluted with an adjuvant or a diluent. Diluents and adjuvants are both well known in the art. An adjuvant is a substance which potentiates the immune response. Specific examples of adjuvants include monophosphoryl lipid A (MPL, SmithKline Beecham); saponins including QS21

(SmithKline Beecham); immunostimulatory oligonucleotides (e.g., CpG oligonucleotides described by Kreig et al., *Nature* 374:546-9, 1995); incomplete Freund's adjuvant; complete Freund's adjuvant; MONTANIDE~~montanide~~; vitamin E and various water-in-oil emulsions prepared from biodegradable oils such as squalene and/or tocopherol, Quil A, MPL and cell wall skeleton from mycobacterium combinations such as ENHANZYN[[^{TM}]] (Corixa Corporation, Hamilton, MT), CRL-1005, L-121, alpha-galactosylceramide (Fujii et al., *J. Exp. Med.*, 2003, Jul. 21; 198(2): 267-79) and combinations thereof. A preferred adjuvant is alum. Other diluents include water suitable for injection, saline, PBS, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethyl formamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan and mixtures thereof.

Please replace the paragraph beginning on page 38, line 30, with the following:

The effect of free amino acids on the dimeric state of rsPSMA (2 mg/ml in PBS+) dialyzed into 20mM sodium acetate and 150mM NaCl at a pH of about 6 was also tested. In general it was found that free amino acids did not have a strong negative effect on dimer association of PSMA and/or column recovery, with the exception of histidine, glutamic acid and aspartic acid used individually at the specific experimental conditions. Therefore, the formulations provided herein can also include a free amino acid or combination of free amino acids, provided that the free amino acid does not have a negative effect that outweighs the dimeric association promoting or preserving nature of the specific formulation. Such free amino acids can be naturally occurring, modified or non-naturally occurring free amino acids (i.e., compounds that do not occur in nature but that can be incorporated into a polypeptide chain; ~~see,~~ for example, ~~http://www.~~cco.caltech.edu/~dadgrp/Unnatstruct.gif~~, which~~ displays structures of non-natural amino acids that have been successfully incorporated into functional ion channels). Modified or non-naturally occurring free amino acids also include but are not limited to 2-aminoadipic acid; 3-aminoadipic acid; beta-alanine, beta-aminopropionic acid; 2-aminobutyric acid; 4-aminobutyric acid, piperidinic acid; 6-aminocaproic acid; 2-aminoheptanoic acid; 2-aminoisobutyric acid; 3-aminoisobutyric acid; 2-aminopimelic acid; 2, 4-diaminobutyric acid;

desmosine; 2,2'-diaminopimelic acid; 2,3-diaminopropionic acid; N-ethylglycine; N-ethylasparagine; hydroxylysine; allo-hydroxylysine; 3-hydroxyproline; 4-hydroxyproline; isodesmosine; allo-isoleucine; N-methylglycine, sarcosine; N-methylisoleucine; 6-N-methyllysine; N-methylvaline; norvaline; norleucine and ornithine. In particular, free amino acids that do not have a negative effect on dimeric association of PSMA and/or column recovery include those that are non-acidic. Examples of these non-acidic free amino acids include glycine, proline, isoleucine, leucine, alanine and arginine.

Please replace the paragraph beginning on page 39, line 21, with the following:

In addition to free amino acids, surfactants and other excipients were also found not to have a negative impact on the dimeric state of PSMA. Therefore, surfactants as well as other excipients can be included in the compositions provided herein. Examples of surfactants include those known in the art and described herein. For example, surfactants include ~~Triton~~TRITON X-100, dodecylmaltoside, cholic acid and CHAPS.

Please replace the paragraph beginning on page 39, line 26, with the following:

Examples of excipients include binders, coatings, compression/encapsulation aids, disintegrants, creams and lotions, lubricants, materials for chewable tablets, parenterals, plasticizers, powder lubricants, soft gelatin capsules, spheres for coating, spheronization agents, suspending/gelling agents, sweeteners and wet granulation agents. Specific examples of such excipients include acetyltriethyl citrate (ATEC); acetyltri-n-butyl citrate (ATBC); aspartame; aspartame and lactose; alginates; calcium carbonate; ~~carbopol~~CARBOPOL; carrageenan; cellulose acetate phthalate-based coatings; cellulose-based coatings; cellulose and lactose combinations; colorants for film coating systems; croscarmellose sodium; crospovidone; dextrose; dibutyl sebacate; ethylcellulose-based coatings; fructose; gellan gum; glyceryl behenate; honey; lactose; anhydrous; lactose; monohydrate; lactose and aspartame; lactose and cellulose; lactose and microcrystalline cellulose; L-HPC (Low-substituted Hydroxy~~Pryopl~~Propyl Cellulose); magnesium stearate; maltodextrin; maltose DC; mannitol DC; methylcellulose-based coatings; microcrystalline cellulose; methacrylate-based coatings; microcrystalline cellulose and carrageenan; microcrystalline cellulose and guar gum; microcrystalline cellulose and lactose;

microcrystalline cellulose and sodium carboxymethylcellulose; molasses DC; polyvinyl acetate phathalate (PVAP); povidone; shellac; sodium starch glycolate; sorbitol, crystalline; sorbitol, special solution; starch DC; sucrose DC; sugar spheres; triacetin; triethylcitrate and xanthan gum. Other excipients include antioxidants and cryoprotectants.

Please replace the paragraph beginning on page 40, line 23, with the following:

For a lyophilized product or a product stored in the cold, one or more cryoprotectants can be added. Typical cryoprotectants for proteins include but are not limited to: sugars such as sucrose, lactose, glucose, trehalose, maltose, and the like; polyols such as inositol, ethylene glycol, glycerol, sorbitol, ~~xylitol~~XYLITOL, mannitol, 2-methyl-2,4-pentane-diol and the like; amino acids such as Na glutamate, proline, alpha-alanine, beta-alanine, glycine, lysine-HCl, 4-hydroxyproline; polymers such as polyethylene glycol, dextran, polyvinylpyrrolidone and the like; inorganics salts such as sodium sulfate, ammonium sulfate, potassium phosphate, magnesium sulfate, and sodium fluoride and the like; organics salts such as sodium acetate, sodium polyethylene, sodium caprylate, proprionate, lactate, succinate and the like; as well as agents such as trimethylamine N-oxide, sarcosine, betaine, gamma-aminobutyric acid, octapine, alanopine, strombine, dimethylsulfoxide, and ethanol.

Please replace the paragraph beginning on page 47, line 28, with the following:

The term "high stringency conditions" as used herein refers to parameters with which the art is familiar. Nucleic acid hybridization parameters may be found in references that compile such methods, e.g. *Molecular Cloning: A Laboratory Manual*, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or *Current Protocols in Molecular Biology*, F. M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. One example of high-stringency conditions is hybridization at 65°C in hybridization buffer (3.5X SSC, 0.02% ~~Ficoll~~FICOLL, 0.02% polyvinyl pyrrolidone, 0.02% Bovine Serum Albumin, 2.5 mM NaH_2PO_4(pH7), 0.5% SDS, 2 mM EDTA). SSC is 0.15M sodium chloride/0.015M sodium citrate, pH7; SDS is sodium dodecyl sulphate; and EDTA is ethylenediaminetetracetic acid. After hybridization, a membrane upon which the nucleic acid is transferred is washed, for

example, in 2X SSC at room temperature and then at 0.1 - 0.5X SSC/0.1X SDS at temperatures up to 68°C.

Please replace the paragraph beginning on page 50, line 22, with the following:

Mammalian lymphocytes typically are immunized by in vivo immunization of the animal (e.g., a mouse) with the desired protein or polypeptide, e.g., with PSMA in the present invention. Such immunizations are repeated as necessary at intervals of up to several weeks to obtain a sufficient titer of antibodies. Once immunized, animals can be used as a source of antibody-producing lymphocytes. Following the last antigen boost, the animals are sacrificed and spleen cells removed. Mouse lymphocytes give a higher percentage of stable fusions with the mouse myeloma lines described herein. Of these, the BALB/c mouse is preferred. However, other mouse strains, rabbit, hamster, sheep and frog may also be used as hosts for preparing antibody-producing cells. See; Goding (in Monoclonal Antibodies: Principles and Practice, 2d ed., pp. 60-61, Orlando, Fla., Academic Press, 1986). In particular, mouse strains that have human immunoglobulin genes inserted in the genome (and which cannot produce mouse immunoglobulins) are preferred. Examples include the ~~HuMAb~~HUMAB mouse strains produced by Medarex/GenPharm International, and the ~~XenoMouse~~XENOMOUSE strains produced by Abgenix. Such mice produce fully human immunoglobulin molecules in response to immunization.

Please replace the paragraph beginning on page 54, line 1, with the following:

Fully human monoclonal antibodies also can be prepared by immunizing mice transgenic for large portions of human immunoglobulin heavy and light chain loci. See, e.g., U.S. Pat. Nos. 5,591,669, 5,598,369, 5,545,806, 5,545,807, 6,150,584, and references cited therein, the contents of which are incorporated herein by reference. These animals have been genetically modified such that there is a functional deletion in the production of endogenous (e.g., murine) antibodies. The animals are further modified to contain all or a portion of the human germ-line immunoglobulin gene locus such that immunization of these animals results in the production of fully human antibodies to the antigen of interest. Following immunization of these mice (e.g., ~~XenoMouse~~XENOMOUSE (Abgenix), ~~HuMAb~~ HUMAB mice (Medarex/GenPharm)),

monoclonal antibodies are prepared according to standard hybridoma technology. These monoclonal antibodies have human immunoglobulin amino acid sequences and therefore will not provoke human anti-mouse antibody (HAMA) responses when administered to humans.

Please replace the paragraph beginning on page 55, line 29, with the following:

The isolated antibody or antigen-binding fragment thereof preferably is selected for its ability to bind live cells expressing PSMA. In order to demonstrate binding of monoclonal antibodies to live cells expressing the PSMA, flow cytometry can be used. For example, cell lines expressing PSMA (grown under standard growth conditions) or prostate cancer cells that express PSMA are mixed with various concentrations of monoclonal antibodies in PBS containing 0.1% ~~Tween~~TWEEN 80 and 20% mouse serum, and incubated at 37°C for 1 hour. After washing, the cells are reacted with fluorescein-labeled anti-human IgG secondary antibody (if human anti-PSMA antibodies were used) under the same conditions as the primary antibody staining. The samples can be analyzed by a fluorescence activated cell sorter (FACS) instrument using light and side scatter properties to gate on single cells. An alternative assay using fluorescence microscopy may be used (in addition to or instead of) the flow cytometry assay. Cells can be stained exactly as described above and examined by fluorescence microscopy. This method allows visualization of individual cells, but may have diminished sensitivity depending on the density of the antigen.

Please replace the paragraph beginning on page 56, line 18, with the following:

The testing of antibody cytolytic activity *in vitro* by chromium release assay can provide an initial screening prior to testing *in vivo* models. This testing can be carried out using standard chromium release assays. Briefly, polymorphonuclear cells (PMN), or other effector cells, from healthy donors can be purified by ~~Ficoll~~FICOLL Hypaque density centrifugation, followed by lysis of contaminating erythrocytes. Washed PMNs can be suspended in RPMI supplemented with 10% heat-inactivated fetal calf serum and mixed with ^{51}Cr labeled cells expressing PSMA, at various ratios of effector cells to tumor cells (effector cells:tumor cells). Purified anti-PSMA IgGs can then be added at various concentrations. Irrelevant IgG can be used as negative control. Assays can be carried out for 0-120 minutes at 37°C. Samples can be assayed for

cytolysis by measuring ^{51}Cr release into the culture supernatant. Anti-PSMA monoclonal antibodies can also be tested in combinations with each other to determine whether cytolysis is enhanced with multiple monoclonal antibodies.

Please replace the paragraph beginning on page 59, line 1, with the following:

Other preferred antibodies include antibodies that specifically (i.e., selectively) bind to an epitope on PSMA defined by a second antibody. To determine the epitope, one can use standard epitope mapping methods known in the art. For example, fragments (peptides) of PSMA antigen (preferably synthetic peptides) that bind the second antibody can be used to determine whether a candidate antibody binds the same epitope. For linear epitopes, overlapping peptides of a defined length (e.g., 8 or more amino acids) are synthesized. The peptides preferably are offset by 1 amino acid, such that a series of peptides covering every 8 amino acid fragment of the PSMA protein sequence are prepared. Fewer peptides can be prepared by using larger offsets, e.g., 2 or 3 amino acids. In addition, longer peptides (e.g., 9-, 10- or 11-mers) can be synthesized. Binding of peptides to antibodies can be determined using standard methodologies including surface plasmon resonance (~~BIACORE~~BIACORE; see Example 22) and ELISA assays. For examination of conformational epitopes, larger PSMA fragments can be used. Other methods that use mass spectrometry to define conformational epitopes have been described and can be used (see, e.g., Baerga-Ortiz et al., *Protein Science* 11: 1300-1308, 2002 and references cited therein). Still other methods for epitope determination are provided in standard laboratory reference works, such as Unit 6.8 ("Phage Display Selection and Analysis of B-cell Epitopes") and Unit 9.8 ("Identification of Antigenic Determinants Using Synthetic Peptide Combinatorial Libraries") of *Current Protocols in Immunology*, Coligan et al., eds., John Wiley & Sons. Epitopes can be confirmed by introducing point mutations or deletions into a known epitope, and then testing binding with one or more antibodies to determine which mutations reduce binding of the antibodies.

Please replace the paragraph beginning on page 61, line 7, with the following:

Agents that act on the tumor vasculature can include tubulin-binding agents such as combrestatin A4 (Griggs et al., *Lancet Oncol.* 2:82, 2001), angiostatin and endostatin (reviewed

in Rosen, *Oncologist* 5:20, 2000, incorporated by reference herein) and interferon inducible protein 10 (U.S. Pat. No. 5,994,292). A number of antiangiogenic agents currently in clinical trials are also contemplated. Agents currently in clinical trials include: 2ME2, Angiostatin, ~~Angiozyme~~ANGIOZYME, Anti-VEGF RhuMAb, Apra (CT-2584), ~~Avicine~~AVICINE, Benefin, BMS275291, Carboxyamidotriazole, CC4047, CC5013, CC7085, CDC801, CGP-41251 (PKC 412), CM101, Combretastatin A-4 Prodrug, EMD 121974, Endostatin, Flavopiridol, Genistein (GCP), Green Tea Extract, IM-862, ImmTher, Interferon alpha, Interleukin-12, ~~Iressa~~IRESSA (ZD1839), Marimastat, ~~Metastat~~METASTAT (Col-3), ~~Neovastat~~NEOVASTAT, Octreotide, Paclitaxel, Penicillamine, ~~Photofrin~~PHOTOFRIN, ~~Photopoint~~PHOTOPOINT, PI-88, Prinomastat (AG-3340), PTK787 (ZK22584), RO317453, Solimastat, Squalamine, SU 101, SU 5416, SU-6668, Suradista (FCE 26644), Suramin (Metaret), Tetrathiomolybdate, Thalidomide, TNP-470 and ~~Vitaxin~~VITAXIN. [[a]] Additional antiangiogenic agents are described by Kerbel, J. Clin. Oncol. 19(18s):45s-51s, 2001, which is incorporated by reference herein. Immunomodulators suitable for conjugation to anti-PSMA antibodies include α-interferon, γ-interferon, and tumor necrosis factor alpha (TNFα).

Please replace the paragraph beginning on page 73, line 8, with the following:

Antigens, such as the PSMA dimers described herein, can be administered with one or more adjuvants to induce or enhance an immune response. An adjuvant is a substance which potentiates the immune response. Adjuvants of many kinds are well known in the art. Specific examples of adjuvants include monophosphoryl lipid A (MPL, SmithKline Beecham); saponins including QS21 (SmithKline Beecham); immunostimulatory oligonucleotides (e.g., CpG oligonucleotides described by Kreig et al., *Nature* 374:546-9, 1995); incomplete Freund's adjuvant; complete Freund's adjuvant; MONTANIDE~~montanide~~; vitamin E and various water-in-oil emulsions prepared from biodegradable oils such as squalene and/or tocopherol, Quil A, Ribi Detox, CRL-1005, L-121, and combinations thereof.

Please replace the paragraph beginning on page 85, line 1, with the following:

Western Blots. Cells were lysed in PBS containing 1mM EDTA, 1% NP-40, 1% TRITON~~Triton~~ X-100, and 5mg/ml aprotinin and cell debris was removed by centrifugation at 3000g for 30 min

at 4°C. Lysates were separated on a 5-20% gradient gel before transfer to nitrocellulose membranes. The resulting blots were blocked in PBS containing 5% milk, 0.02% SDS and 0.1% TRITON~~Triton~~ X-100 before incubation with MAB544 primary antibody (Maine Biotechnologies) at a concentration of 2mg/ml. After three washes, blots were incubated with a goat anti-mouse HRP-conjugated secondary antibody at a concentration of 0.2mg/ml. Blots are visualized using the Renaissance chemiluminescence system (Perkin-Elmer Life Sciences, Boston, MA).

Please replace the paragraph beginning on page 85, line 11, with the following:

ELISA. Cells were lysed in PBS containing 1mM EDTA, 1% NP-40, 1% TRITON~~Triton~~ X-100, and 5mg/ml aprotinin. The resulting cell membranes were plated onto 96-well plates and dried in a sterile hood overnight. The plates were then blocked with PBS containing casein and TWEEN ~~Tween~~-20 before addition of mouse sera or hybridoma supernatants, using purified MAB544 (Maine Biotechnologies) or 7E11 (Cytogen) as a standard. After washing in PBS, an alkaline phosphatase conjugated secondary antibody (subclass specific) was incubated and subsequently washed in PBS. The pNPP substrate was then added for colorimetric detection at a wavelength of 405 nm.

Please replace the paragraph beginning on page 86, line 6, with the following:

Mice having the ability to produce human antibodies (~~XenoMouse~~XENOMOUSE, Abgenix; Mendez et al., *Nature Genetics* 15:146, 1997) were immunized subcutaneously once or twice weekly with 5 X 10^6 LNCaP cells adjuvanted with alum or TITERMAX~~Titermax~~ Gold (Sigma Chemical Co., St. Louis, Mo.). Animals were boosted twice with 10 μg of recombinant PSMA protein immunoaffinity captured onto protein G magnetic microbeads (Miltenyi Biotec, Auburn, CA). PSMA mAb 3.11 was used for capture. Splenocytes were fused with NSO myeloma cells and the hybridomas that resulted were screened as above by flow cytometry to detect clones producing antibodies reactive with the extracellular portion of PSMA. One clone, 10.3 (PTA-3347), produced such antibodies.

Please replace the paragraph beginning on page 88, line 19, with the following:

Culture supernatants from a given hybridoma are pooled and loaded onto a Protein A SEPHAROSE~~Sepharose~~ affinity column. Mouse IgG2a, mouse IgG2b and human IgG1 antibodies are loaded directly, but supernatants containing mouse IgG1 antibodies are adjusted to pH 8.5 and 1M NaCl prior to loading in order to promote binding. After washing the column, the mAb is eluted with low pH buffer into fractions using 1M Tris, pH 8.0. Elution peak fractions are pooled, dialyzed against PBS buffer, concentrated to 5 mg/mL and stored in sterile aliquots at -95°C. All purification procedures are carried out using endotoxin-free buffers and sanitized chromatography columns. Purified mAbs are tested for purity by reducing and nonreducing SDS-PAGE, for PSMA binding affinity by ELISA, and for endotoxin levels by the *limulus* amebocyte lysate assay. These procedures routinely yield "animal-grade" antibody at >95% purity and <0.5 endotoxin units per milligram of protein.

Please replace the paragraph beginning on page 89, line 12, with the following:

For ELISA, CHO cell-derived recombinant soluble PSMA protein (rsPSMA, Progenics, Tarrytown, NY) is diluted to 1 µg/ml in 50 mM carbonate buffer, pH 9.4, and coated overnight at 4 °C onto 96-well IMMULON~~Immulon~~ II microtiter plates at 100 µl/well. The plates are then blocked for 2 hr with PBS buffer containing 5% BSA. mAbs are added in a range of concentrations in ELISA buffer (PBS buffer containing 2% BSA, 1% FBS and 0.5% TWEEN~~Tween~~ 20) for 2 hours at room temperature. The plates are washed, and horseradish peroxidase conjugated goat antibody to mouse IgG is added for 1 hr at room temperature. The plates are washed again and 3,3',5,5'-tetramethylbenzidine dihydrochloride (TMB) substrate (Pierce, Rockford, IL) is added for colorimetric readout at 450 nm using an ELISA plate reader (Molecular Devices, Sunnyvale, CA).

Please replace the paragraph beginning on page 91, line 11, with the following:

Anti-PSMA mAbs are tested by immunohistochemistry for reactivity with both normal and malignant human tissues using an avidin-biotin peroxidase method (Silver, D. A. *et al. Clin Cancer Res* 3: 81-85, 1997). Frozen or paraffin-embedded tissues can be used. Paraffin-embedded tissue sections are deparaffinized and endogenous peroxidase activity is blocked by

incubation with 1% H_2O_2 for 15 minutes. Sections are blocked in a 1:10 dilution of horse serum in 2% PBS-BSA (Sigma Chemical, St Louis, Mo.) for 30 minutes before overnight incubation with 2μg/ml anti-PSMA mAb in 2% PBS-BSA. After washing, sections are incubated with biotinylated secondary antibody, washed, and incubated with avidin:biotin peroxidase complexes (Vector Laboratories, Burlingame, CA) diluted 1:25 in PBS for 30 minutes. After washing, sections are visualized by immersion in PBS containing 0.05% diaminobenzidine tetrachloride, 0.01% H_2O_2, and 0.5% TRITON~~Triton~~ X-100. Negative control sections are incubated with isotype-matched mAbs of irrelevant specificity. As a positive control, 7E11 (Cytogen, Princeton, NJ), a well-characterized anti-PSMA mAb, is used.

Please replace the paragraph beginning on page 98, line 11, with the following:

Four to six week old nude athymic BALB/c male mice are obtained from the National Cancer Institute-Frederick Cancer Center and maintained in pressurized ventilated caging. While immunodeficient in many respects, these mice mediate wild-type levels of ADCC and CML. The CWR22 tumor line is propagated in the animals by the injection of minced tumor tissue from an established tumor into the subcutaneous tissue of the flanks of athymic nude mice together with reconstituted basement membrane (MATRIGEL~~Matrigel~~, Collaborative Research, Bedford, MA). To maintain serum androgen levels, the mice are administered 12.5-mg sustained-release testosterone pellets (Innovative Research of America, Sarasota, FL) subcutaneously before receiving tumors. Three to four weeks after inoculation, tumors of approximately 1.5 x 1.0 x 1.0 cm are measured. Androgens are withdrawn by surgical castration under pentobarbital anesthesia and removal of the sustained-release testosterone pellets. Tumor size is determined by caliper measurements of height, width and depth. PSA values are performed on the serum of the mice after tail bleeding using a Tandem-R PSA immuno-radiometric assay (Hybritech, San Diego, CA).

Please replace the paragraph beginning on page 99, line 19, with the following:

The extracellular domain of PSMA (amino acids 44-750 of the full-length protein, SEQ ID NO:1) was obtained as a secreted protein from a DXB11 Chinese hamster ovary (CHO) cell line, stably transfected with a[[an]] rsPSMA expression vector. The cells were grown in a

Celligen Plus 2.2L Packed Bed Bioreactor (New Brunswick Scientific, Edison, NJ) in protein-free media. The Bioreactor was operated in perfusion mode, and supernatant was collected aseptically into collection bags maintained at 4°C. The protease inhibitor aprotinin was added to the harvest supernatant, which was concentrated 25-fold prior to storage at -90°C. In some instances for purification, the concentrate was thawed and purified using subsequent steps of Concanavalin A lectin affinity chromatography and Butyl-SEPHAROSE~~Sepharose~~ hydrophobic interaction chromatography or according to the steps shown below.

Please replace the paragraph beginning on page 100, line 3, with the following:

Cell culture supernatants were concentrated 25-fold by tangential flow ultrafiltration and adjusted to 35% saturation with ammonium sulfate. Under these conditions, rsPSMA remains in the supernatant. Precipitated proteins were removed by centrifugation (20,000 x *g* for 30 min, SS-34, Sorvall, Inc.) and the clarified supernatant was applied to a Butyl-SEPHAROSE~~Sepharose~~ resin (BioRad, Hercules, CA) followed by a wash with 35% ammonium sulfate in neutral phosphate-buffered saline containing 1mM Ca^{2+} and 0.5mM Mg^{2+} (PBS+). rsPSMA eluted in the flow-through and wash fractions of the column. The fractions containing the rsPSMA protein were pooled, dialyzed into 10mM sodium phosphate, pH 7.0, and loaded onto a Ceramic Hydroxyapatite column (BioRad, Hercules, CA). rsPSMA was eluted from the resin using 2M sodium chloride in 10mM sodium phosphate, pH 7.0. The fractions containing the protein were pooled, dialyzed into 20mM Tris, pH 7.5 containing 1mM Ca^{2+} and 0.5mM Mg^{2+}, and applied to a Q650-SEPHAROSE~~Sepharose~~ column (TosoHaas, Montgomeryville, PA). rsPSMA was eluted from the resin with 150mM NaCl in 20mM Tris, pH 7.5 containing 1mM Ca^{2+} and 0.5mM Mg^{2+}. Monomeric and dimeric forms of rsPSMA present after this step were separated using preparative size exclusion chromatography on a ~~Superdex~~SUPERDEX 200 resin (Amersham Biosciences, Piscataway, NJ) and PBS+ (containing 1mM Ca^{2+} and 0.5mM Mg^{2+}) as the running buffer. Purified rsPSMA was stored at -80°C in PBS+. Unless otherwise indicated, PSMA monomers represent spontaneously dissociated protein recovered over SEC rather than forcibly denatured material.

Please replace the paragraph beginning on page 101, line 9, with the following:

Western blotting was performed as follows: Subsequent to transfer, the nitrocellulose membrane was blocked with 5% milk in PBS/0.1% TRITON~~Triton~~ X-100/0.02% SDS, which was also used for the subsequent wash and antibody incubation steps. PSMA proteins were detected using the anti-PSMA mAbs 3.1 or 3.9 (Progenics Pharmaceuticals) as primary antibody and HRP-labeled anti-mouse IgG as secondary antibody and 1h incubation at room temperature. The membranes were colorimetrically developed using chemiluminescence (NEN Plus, Perkin Elmer Life Sciences, Boston, MA).

Please replace the paragraph beginning on page 106, line 24, with the following:

Flow cytometry data (mean fluorescence intensity v. antibody concentration) were transposed and plotted using EXCEL~~Excel~~ software (Microsoft, Redmond, WA). Results from representative experiments of at least three determinations are depicted in Figs. 28A-28C. Binding was compared by calculation of 50% effective concentration (EC50) using the Forecast function in ~~Excel~~EXCEL. The EC50 value represents the concentration of antibody required for half-maximal binding.

Please replace the paragraph beginning on page 107, line 19, with the following:

Murine 3.9, 5.4, mJ591 (ATCC# HB-12126) and human 006, 4.40, 4.304 anti-PSMA antibodies (and control IgG antibodies) were added into plates at different concentrations to bring the total volume to 200 μL in triplicate. The plates were kept cold on ice for at least 30 min to maximize Map-Zap or Hum-Zap binding to PSMA antibodies before internalization. The plates were incubated for 2 days and then the medium was changed and incubated for another 2 days. After 4 days incubation, the medium was withdrawn and fresh medium containing 10% Alamar Blue (20 μL, Bioscience, Camarillo, CA) was added into each well and incubated for 2 hrs. A ~~CytoFlour~~CYTOFLUOR plate reader was used to measure fluorescence in 96-well plates at wavelengths of 530 nm excitation and 590 nm emission. Internalization of toxin was mediated by anti-PSMA antibodies. The cell kill is illustrated in Fig. 29 on C4-2 cells and in Fig. 30 on PSMA-3T3 cells.

Please replace the paragraph beginning on page 110, line 8, with the following:

Example 22: Binding Affinity Using ~~Biacore 3000~~BIACORE 3000

Please replace the paragraph beginning on page 110, line 9, with the following:

To determine the kinetics and affinity of the antibodies, the antibodies in crude supernatants, in purified form and in bifunctional chelate modified forms were analyzed using a ~~Biacore 3000~~BIACORE 3000 instrument (Biacore Inc., Piscataway, NJ). ~~Biacore 3000~~BIACORE 3000 is a fully automated surface plasmon resonance (SPR)-based biosensor system that is designed to provide real-time kinetic data from assay formats that require no tags or labeling of compounds for biomolecular interactions. It is ideal for screening crude supernatants.

Please replace the paragraph beginning on page 110, line 15, with the following:

The streptavidin-coated sensor chips (SA chips, Biacore, Inc.) were used to capture biotinylated anti-human IgG antibody (Sigma, St. Louis, MO). The entire sensor chip surface was conditioned with five injections of conditioning solution (1 M NaCl, 50 mM NaOH) and equilibrated with PBS buffer containing 0.005% polysorbate 20. Two to three thousand resonance units (RU) of biotinylated anti-human IgG antibody (Sigma) were immobilized onto the SA chip followed by an injection of regeneration buffer (glycine-HCl, pH 2.2). Antibodies in supernatants were diluted to 2 μg/mL in PBS buffer and captured onto one anti-human IgG flow cell, while isotype-matched control human antibody (Sigma) was similarly captured on a second flow cell. rsPSMA at different concentrations in PBS buffer was flowed over the cells at 30 μL/min for 3 min in an "association phase" followed by a "dissociation phase" for 10 min. SPR was monitored and displayed as a function of time. For each antibody at one concentration, the chip was regenerated and equilibrated. Examples of the analysis of antibody PRGX1-XG-006 in association phase and dissociation phase at different concentrations of rsPSMA from 100 nM to 6.25 nM are shown in Fig. 34. Thermodynamic and kinetic rate constants of binding were calculated using the ~~Biacore~~BIACORE Evaluation software. For example, the affinity of XG-006 antibodies in a supernatant to rsPSMA was determined to be 4.92×10^{-10} M with a K_a of 1.3×10^5 M^{-1} s^{-1} and a K_d of 6.4×10^{-5} s^{-1}. Selective data for several human PSMA antibodies in

crude supernatant, purified form, and modified with bifunctional chelate is listed in Table 5 for comparison.

Please replace the paragraph beginning on page 111, line 12, with the following:

A comparison of the fully human antibodies 4.40.1, 4.49.1, 051 and 006 and the murine antibody 3.9 was performed by ~~Biacore~~BIACORE. For each antibody for comparison, response was normalized to 100 RU. The graph of time vs. response difference for these antibodies is given in Fig. 35. The binding affinities for these antibodies were determined to be 6.1, 6.7, 5.8, 4.8 and 13.7×10^{-10}M, respectively.

Please delete Table 6, beginning on page 112, line 12, and replace with the following:

	Ab Conc (μg/mL)		Binding to 3T3-PSMA (FACS)					~~Biacore~~BIACORE studies		
Supernatant	PGNX	Lysate EIA	PGNX FACS	AVG Max binding	AVG EC50	C4.2 FACS	Anti-PSMA Western	KD, M-1 (x 10^{-10})	Ka, M-1s-1 (x 10^{5})	Kd, s-1 (x 10-5)
PRGX1-XG1-026	4.7	ND[1]	ND	148	2.4	ND	Conf.[2]	2.0	1.5	2.9
4.4.1	4.7	0.08	7	8	ND	5.2	Conf.	4.2	2.3	9.7
PRGX1-XG1-006	1.8	0.39	114	183	3.4	9.5	Conf.	4.8	1.3	6.4
PRGX1-XG1-051	3.5	0.48	83	202	2.0	9.9	Conf.	5.8	1.4	8.2
4.40.1	4.3	0.33	53	163	2.3	10.8	Conf.	6.1	2.1	12.5
4.49.1	2.6	0.36	362	162	0.9	16.2	Conf.	6.7	3.1	20.7
4.292.1	2.7	0.18	75	195	6.0	9.2	Conf.	6.8	1.2	8.5
4.304.1	4.1	0.39	92	184	9.1	8.4	Conf.	8.7	1.4	12.5
4.232.1	2.4	0.49	97	138	2.7	6.0	Linear[3]	9.4	1.5	13.8
4.153.1	5.9	0.29	279	182	5.3	14.8	Conf.	9.5	1.2	11.8
4.333.1	2.9	0.18	82	168	3.1	6.6	Conf.	11	0.7	8.5
PRGX1-XG1-077	3.9	0.45	392	227	6.0	12.4	Conf.	16	0.6	10.4
10.3	8.5	1.06	ND	ND	ND	ND	ND	19	1.9	36.4
pure 10.3		0.44	130	181	7.5	ND	Conf.	ND		
						4.7				
4.22.1	2.8	0.08	7	ND	ND	4.7	ND	*20*	*1.7*	*33*
4.248.1	3.5	0.37	7	ND	ND	4.1	Conf.	*27*	*1.0*	*28*
4.54.1	10	0.14	267	162	3.9	13.6	ND	30	1.9	56
4.7.1	5	0.23	156	141	1.6	10.2	Conf.	32	1.7	56
4.78.1	5.3	0.00	205	118	1.0	7.9	Conf.	*53*	*2.4*	*125*
4.48.1	4.9	0.06	14	ND	ND	7.7	ND	*62*	*0.9*	*59*
4.209.1	3.5	0.22	60	ND	ND	6.7	ND	*142*	*0.9*	*125*
4.177.1	1.1	0.15	236	174	2.4	10.6	ND	*155*	*0.6*	*93*
4.152.1	3.4	0.38	81	85	4.0	7.5	ND	*163*	*0.8*	*126*
4.28.1	4.2	0.04	112	155	4.2	11.3	ND	*167*	*1.2*	*192*
4.16.1	5.3	0.00	8	ND	ND	7.8	ND	*177*	*1.8*	*313*
4.360.1	1.5	0.02	112	130	2.2	7.9	ND	*197*	*1.0*	*201*
4.288.1	15.4	0.02	67	141	4.1	6.5	ND	*198*	*1.3*	*257*
4.219.2	0.5	0.34	69	ND	ND	5.9	ND	ND		
PRGX1-XG1-069	6.5	ND	ND	71	7.9	ND	ND	No Binding		
Murine 3.9								13.7	0.7	9.7
Control								6.34	2.24	14.2

[1] ND=not determined
[2] conf.=conformational epitope
[3] linear=linear epitope

Please replace the paragraph beginning on page 116, line 19, with the following:

A ~~Biacore 3000~~BIACORE 3000 instrument was used to monitor, in real time, binding of rsPSMA dimer and monomer to anti-PSMA mAbs. Antibodies were immobilized at approximately 10,000 resonance units to CM5 sensor chips according to the manufacturer's instructions for amine coupling (Biacore, Inc., Piscataway, NJ). A reference surface of isotype-matched antibody of irrelevant specificity was used as a background control. Binding experiments were performed at 25°C in PBS buffer with 0.005% [vol/vol] Surfactant P20. Purified rsPSMA dimer (50 nM) or monomer (100 nM) was passed over control and test flow cells at a flow rate of 5 μL/min. The sensor surface was regenerated with two pulses of 20 nM HCl.

Please replace the paragraph beginning on page 117, line 7, with the following:

BALB/c mice were immunized by subcutaneous injection at days 0, 7, 14, and 42 with either 5 μg clinical rsPSMA lot # 4019-C001 (75 % dimer/25 % monomer) or 5 μg rsPSMA batch # TD045-003 run 1/peak 2 (100 % monomer) on alum (250 μg per dose, Sigma) or adjuvanted with 50 μg ALHYDROGEL~~alhydrogel~~ per dose. Serum was drawn 10 days after the fourth immunization and analyzed by enzyme-linked immunoassay (EIA) and flow cytometry.

Please replace the paragraph beginning on page 117, line 14, with the following:

rsPSMA lot # 4019-C001 or rsPSMA batch # TD045-003 run 1/peak 2 was passively adsorbed to 96-well microtiter plates. Remaining binding sites on the plate were blocked with a PBS/Casein/~~Tween~~ TWEEN 20 buffer. Serially diluted mouse serum or controls were added and bound antibody was detected using a goat anti-mouse IgG antibody conjugated to alkaline phosphatase. The EIA was developed with the substrate pNPP which produces a color change that is directly proportional to the amount of anti-PSMA antibody bound. Absorbance was read at 405 nm with a correction of 620 nm. Antibody titer was defined as the highest dilution of mouse serum yielding a blank corrected absorbance of 0.1. Immune mouse serum with a known anti-PSMA titer or normal mouse serum with no anti-PSMA reactivity was used as controls.

Please replace the paragraph beginning on page 118, line 15, with the following:

Table 7: Specificity of the Anti-PSMA Antibody Response in Mice Vaccinated 4 Times with rsPSMA 5 µg/dose and 50 µg/dose ~~Alhydrogel~~ALHYDROGEL

Please replace the paragraph beginning on page 123, line 6, with the following:

Dimeric rsPSMA (2 mg/ml in PBS+) was diluted 10-fold into PBS+ containing 0.5% (w/v) of either TRITON~~Triton~~ X-100, dodecylmaltoside, cholic acid, or CHAPS and incubated for 4 days at 4°C. Each sample was subsequently analyzed by analytical TSK gel filtration chromatography for protein recovery and the preservation of the dimeric structure of the protein. The findings are summarized in Table 11.

Please replace Table 11, at the paragraph beginning on page 123, line 11, with the following:

Table 11: Recovery and Structure of rsPSMA with Various Surfactants

Surfactant	Dimer Content[1]	Monomer Content[1]	Aggregate Content[1]	Recovery from column[2]
TRITON~~Triton~~ X-100	++++	+	+	++++
Dodecylmaltoside	++++	-	+	+++++
Cholic Acid	++++	-	+	+++++
CHAPS	++++	+	-	+++++

[1] of recovered protein [2] of total protein at t=0

- <5% + 5%-25% ++ 25-50% +++ 50-75% ++++ 75-95% +++++ >95%

Please remove the Sequence Listing mailed on August 16, 2007 and enter the attached Substitute Sequence Listing in its place.